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STANDARDS OF PURITY FOR WATER USED IN RAILROAD COACHES[1]

By Zelma Zentmire

Standards of purity of water depend to a great extent upon methods of bacteriological analysis. Bacteriologists are constantly attempting to improve the methods in use, more especially procedures for the detection of sewage organisms. The water-borne diseases most dreaded in America are typhoid fever and dysentery. The causative organisms are very difficult to recognize in water, hence search is made for the more easily detected colon group always present in fecal material. Organisms of this group produce gas and acid in suitable culture media.

The methods approved by the Treasury Department, United States Public Health Service, are in many respects like those adopted by the American Public Health Association. Both use 1 per cent lactose in broth and in lactose agar. The reaction is adjusted by titration, and lactose is added before sterilization. The litmus lactose agar contains 1.2 per cent agar. The test for gas formation is made by planting lactose broth tubes with definite amounts of water and by fishing acid colonies from a plate to a tube of broth. The test for acid production consists in inoculating plates of either litmus lactose agar or of Endo's medium, with material from a broth tube which shows the presence of gas formers. According to the methods of the American Public Health Association, lactose broth tubes showing at least 10 per cent of gas after twenty-four hours incubation should be tested immediately for acid formation; otherwise, continue the incubation for an additional twenty-four hours, when gas production in any amount requires a test for acid formation. The Public Health Service requires that only tubes showing more than 5 per cent of gas after forty-eight hours incubation be tested for acid producers. The aim of this paper is to review briefly some of the more recent experimental work bearing on the detection of sewage organisms. The discussion falls naturally into six divisions.

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[1] Read before the Iowa Section, April 16, 1919.

1. Recognition of the colon group. Meyer[2] advocates the use of litmus lactose agar containing 3 per cent of agar. He found that this concentration of agar inhibited the development of organisms outside the colon group. This medium compared favorably with the standard Endo medium. Hasseltine[3] finds that an Endo containing five times as much indicator as the standard Endo gives many more typical colonies, and restricts other bacteria to a greater extent. Ayrs and Rupp[4] prevent the development of the majority of other bacteria by the use of a modified Endo of such simple ingredients that practically only members of the colon group thrive on it. Kahn[5] finds the standard Endo satisfactory if used in a test tube. From a lactose broth culture a smear is made on the surface of the Endo slant. A stab culture made by inserting the wire to the butt of the tube shows reddening of the medium and gas bubbles if the colon group is present. The advantage lies in the saving of time and material due to the production of typical colonies on the slant and the simultaneous development of gas and acid in the butt of the tube. Kahn[6] in his work in the southeastern area of the United States found that this medium prevented the development of gas and acid formers outside of the colon group. Hall and Ellefson[7] use the dye gentian violet in lactose broth and in litmus lactose agar to inhibit to a very large extent all organisms except those of the colon group. Much time and material are saved by preventing the formation of gas by many organisms which are of no sanitary significance, yet are frequently present in water samples.

2. Effect of sterilization upon lactose in culture media. Lactose is used in the medium for detection of gas formers because it is fermented by the members of the colon group, though not by many other bacteria of little sanitary significance. Under certain conditions it can be broken down into two simple sugars, galactose and dextrose. Dextrose is fermented by numerous organisms, hence is undesirable in a medium for the detection of the colon group.

The most common method for the sterilization of a medium is to subject it to the action of steam under conditions of increased temperature and pressure, the autoclave method. Another method is to heat the medium in an Arnold sterilizer, which subjects it to the action of steam without increased pressure. Hasseltine[3] finds that sterilization in the Arnold breaks down lactose to a smaller extent than does autoclaving; Mudge[8] obtained results which indicate that the opposite is the case. He advises sterilization of sugar broth by filtration through unglazed porcelain. Levine[9] adds to melted solid media previously sterilized in the autoclave, either powdered lactose or lactose solution sterilized by heat.

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[2] Meyer, 1917, *Jour. of Bacteriology*, ii, 237.

[3] Hasseltine, 1917, *U. S. P. H. S., Reports*, xxxii, no. 45.

[4] Ayers and Rupp, 1918, *Jour. of Bacteriology*, iii, 433.

[5] Kahn, 1918, *Jour. of Bacteriology*, lii, 555.

[6] Kahn, 1918, *Jour. of Bacteriology* iii, 561.

[7] Hall and Ellefson, 1918, *Jour. of Bacteriology*, iii, 329.

3. Reaction of media. It is customary to measure the reaction of media by determining the titratable acidity before sterilization. Mudge[8] and Norton[9] report that sterilization of sugar medium by heat produces acid except where the medium is already distinctly acid. It has been demonstrated that the hydrogen ion concentration is a true measure of the reaction. The hydrogen ion concentration can be accurately determined electrometrically, but for routine work a simple colorimetric method is sufficiently accurate. Clark and Lubs[10] advocate a colorimetric procedure for routine work. Two modifications to simplify the method have been proposed.[11, 12]

4. Time required to conplete a test. For obvious reasons the length of time required to determine the quality of a water is of considerable importance. Usually from forty-eight to ninety-six hours are required to prove the presence or absence of the colon group. Hall and Ellefson[7] conclude that neither the time of the appearance of gas nor its quantity gives a clue to the nature of the gas former, and that one is justified in waiting at least four days before saying gas formers are absent. Others[13] also find that the amount of gas produced is of no significance. Burling and Levine[14] find a 0.5 per cent concentration of lactose in lactose broth most satisfactory, because as the concentration of the sugar increases the longevity of the bacteria decreases. They determined acid production by gas formers as quickly as possible, preferably in from twelve to twenty-four hours after inoculation of the broth. Bronfenbrenner and Davis[15] report that more than 1 per cent lactose may greatly hasten the production of gas when acid colonies are fished from a plate. In this case, however, gas formers have already been shown to be acid producers, hence there is no object in reducing the sugar concentration to prevent death of the organisms. Kahn[5] makes inoculations into his Endo tubes after about 15 hours incubation in lactose broth, even though there is no sign of gas production. In the presence of the colon group, continued incubation of the broth usually shows gas formation simultaneously with the production of typical colonies on the Endo slant and gas and acid production in the butt. Hence he completes the test in a comparatively short time.

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[8] Mudge, 1917, *Jour. of Bacteriology,* ii, 403.

[9] Levine, (a) 1918, *Jour. of Infectious Diseases,* xxiii, 43; (b) 1918, *American Jour. of Public Health,* viii, 3.

[10] Clark and Lubs, 1917, *Jour. of Bacteriology,* ii, 1–34, 109–136, 191–236.

[11] Barnett and Chapman, 1918, *Jour. American Medical Association,* lxx, 1062.

[12] Norton, 1919, *Amer. Jour. of Public Health,* ix, 190.

[13] Browne and Ackman, 1917, *Jour. of Bacteriology,* ii, 249.

[14] Burling and Levine, 1918, *Amer. Jour. Public Health,* viii, 306.

5. Separation of the colon group into organisms of different sanitary significance. It is now accepted as a fact that the colon group includes bacteria of widely different sanitary significance. The colon bacilli are found chiefly in the feces of man and warm blooded animals, and Bacillus aerogenes chiefly on grains and in cropped soils. Levine[9] has modified two media so that it is possible to distinguish between colonies of B. coli and B. aerogenes on a plate inoculated with material from a lactose broth culture.

6. Significance of gas formers which do not show acid production. When gas formers are present in a water sample and the analyst is unsuccessful in demonstrating that they are acid formers, it has been suggested that one or more of the following conditions may exist:

1. Organisms which produce gas under anaerobic conditions, but no acid under aerobic conditions, are present.[7, 16]
2. Members of the group have died in the enrichment culture.[14]
3. Proteolytic organisms may liberate enough alkali to neutralize any acid produced.[7]
4. It may be possible that so-called "attenuated" organisms of the colon group have partially or temporarily lost their property of fermenting lactose under aerobic conditions.[7]

A study of the data secured during the past eight months in the examination of water samples from all parts of Iowa may be of interest in the light of the above discussion. There have been considered here only samples from actual drinking supplies, i.e., samples which were not grossly polluted. The data recorded were secured from 211 samples showing gas formers and analyzed according to the 1917 Standard Methods of the American Public Health Association. Nearly all were ground waters, the majority of which were untreated. Ninety-six samples analyzed according to the method of the American Public Health Service contained gas formers. These samples were practically all treated surface waters. The test for acid production was made on litmus lactose agar plates.

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[15] Bronfenbrenner and Davis, 1918, *Jour. Med. Research,* xxxix, 33.

[16] Creel, 1914, *U. S. P. H. S., Hygienic Lab. Bull. No.* **100**, 43.

TABLE 1

Relation of acid production to time of appearance of gas and quantity of gas

	TIME OF APPEARANCE OF GAS				GAS UNDER 10 PER CENT			
	24 hours		48 hours		24 hours		48 hours	
	10 cc.	1 cc.	10 cc.	1 cc.	10 cc.	1 cc.	10 cc.	1 cc.
A. P. H. A. method:								
Number of tubes gas-formers	47	74	77	57				
Number of tubes acid-formers	35	56	8	17	10	8	0	4
Percentage of tubes acid-formers	74	76	10	30	21	11	0	4
					GAS UNDER 6 PER CENT			
U. S. P. H. S. method:								
Number of tubes gas-formers	9	0	205	4				
Number of tubes acid-formers	3	0	50	2	3	0	8	0
Percentage of tubes acid-formers	33	0	24	50	33	0	4	0

Inspection of the table indicates that in the first class of samples acid formers were present in three-fourths of the tubes showing gas formers at the end of twenty-four hours incubation, while a much smaller percentage of gas formers were acid formers if there was no gas at twenty-four hours. A tenth to a fifth of the acid formers showed less than 10 per cent gas at twenty-four hours. Less than 10 per cent gas in forty-eight hours rarely indicated acid formers.

In the second class of samples, gas seldom appeared with twenty-four hours incubation. If absent at twenty-four hours but present at forty-eight hours, only one-fourth of such tubes contained acid formers. Four per cent of the acid formers were gas formers with less than 6 per cent gas at the end of forty-eight hours.

It is obvious that the recognition of bacteria indicative of sewage contamination is dependent upon a great variety of factors. Some of these factors are well known and easily controlled; the importance of others is not as yet definitely determined, and still others are rather difficult to control by methods known at present. A great deal of work is still necessary before we have a bacteriological procedure for the recognition of sewage pollution of water which may be regarded as a standard method on which complete dependence may be placed.